The influence of catabolic reactions on polyamine excretion

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Complete inhibition of polyamine catabolism is possible by combined administration of two compounds. Aminoguanidine (25 mg/kg body wt., intraperitoneally) inhibits all reactions that are catalysed by copper-containing amine oxidases (CuAO). The products of the CuAO-catalysed reactions cannot be reconverted into polyamines (terminal catabolism) and therefore usually escape observation. *N*¹-Methyl-*N*²-(buta-2,3-dienyl)butane-1,4-diamine (MDL 72521) is a new inhibitor of polyamine oxidase. It inhibits completely the degradation of *N*¹-acetylspermidine and *N*¹-acetylspermine. The enhanced excretion of *N*¹-acetylspermidine in urine after administration of 20 mg of MDL 72521/day per kg body wt. is a measure of the rate of spermidine degradation *in vivo* to putrescine, and thus of the quantitative significance of the interconversion pathway. From the enhancement of total polyamine excretion by aminoguanidine-treated rats, one can calculate that only about 40% of the polyamines that are destined for elimination are usually observed in the urine, the other 60% being catabolized along the CuAO-catalysed pathways. The normally observed urinary polyamine pattern gives, therefore, an unsatisfactory picture of the actual polyamine elimination. Although aminoguanidine alone is sufficient to block terminal polyamine catabolism, rats that were treated with a combination of aminoguanidine and MDL 72521 excrete more polyamines than those that received aminoguanidine alone. The reason is that a certain proportion of putrescine, which is formed by degradation of spermidine, is normally reutilized for polyamine biosynthesis. In MDL 72521-treated animals this proportion appears in the urine in the form of *N*¹-acetylspermidine. Thus it is possible to determine polyamine interconversion and re-utilization *in vivo* and to establish a polyamine balance in intact rats by using specific inhibitors of the CuAO and of polyamine oxidase.

If we disregard acetylputrescine, whose degradation by monoamine oxidase (EC 1.4.3.4) is assumed to be of physiological importance in brain (Seiler & Al Therib, 1974), the polyamines and their natural monoacetyl derivatives are degraded in vertebrates by two types of enzymes.

(a) Polyamine oxidase (FAD-dependent) (PAO) has been localized in liver peroxisomes (Hölttä, 1977) and is present virtually in all tissues (Seiler *et al.*, 1980). Evidence has accumulated showing that this enzyme is involved in the interconversion of

polyamines (Seiler, 1981; Bolkenius & Seiler, 1981; Seiler *et al.*, 1981a).

(b) Probably several copper-containing amine oxidases (CuAO) exist that are capable of oxidatively deaminating diamines or polyamines and their natural monoacetyl derivatives. The best known enzymes among these are diamine oxidase (EC 1.4.3.6) and bovine serum amine oxidase (Hirsch, 1953). Hog kidney diamine oxidase (Suzuki *et al.*, 1981), human pregnancy serum (Gahl & Pitot, 1981), and foetal-calf and bovine serum (Gahl & Pitot, 1982) are capable of oxidizing not only the diamines and spermidine and spermine, but also their monoacetyl derivatives. More importantly, *N*¹-acetylspermidine is a natural substrate of a CuAO (Seiler *et al.*, 1983), and the oxidative deamination of putrescine, spermidine, spermine and of *N*¹-acetylspermidine

Abbreviations used: CuAO, copper-containing amine oxidase; MDL 72521, *N*¹-methyl-*N*²-(buta-2,3-dienyl)-butane-1,4-diamine dihydrochloride (*N*¹-methyl-*N*²-allenylputrescine); PAO, polyamine oxidase; AG, aminoguanidine sulphate; DFMO, α -DL-difluoromethylornithine; ODC, ornithine decarboxylase.

Scheme 1. *Catabolic pathways of the natural polyamines*

Compounds in boxes have been found as normal constituents of the urine of rodents (Nakajima *et al.*, 1980; Seiler & Knödgen, 1983; Seiler *et al.*, 1982). The 'squiggly' arrow indicates the rate-limiting step that is catalysed by acetyl-CoA:polyamine *N*¹-acetyltransferase (Pegg *et al.*, 1982).

can be completely inhibited in rats or mice if these animals are pretreated with aminoguanidine sulphate (AG) at a dose in excess of 20 mg/kg body wt. (Seiler *et al.*, 1982; Seiler & Knödgen, 1983; Seiler *et al.*, 1983). In contrast with PAO and monoamine oxidase, which are more or less evenly distributed in the body, CuAO enzymes are unevenly distributed in the vertebrate organism. The major source of the diamine (Shaff & Beaven, 1976)- and polyamine (Seiler *et al.*, 1983)-degrading activity seems to be localized in the small intestines.

Scheme 1 illustrates the catabolic relationships between the polyamines. CuAO transform the polyamines into compounds that cannot be reconverted into polyamines. Therefore we may summarize these reactions under the term 'terminal catabolism'. In contrast, PAO transforms one polyamine into another, as was mentioned above.

Recently MDL 72521 became available. This compound is a potent and specific irreversible inhibitor of pig and rat liver PAO (K_i 0.3 μ M; $\tau_{1/2}$ = 0.5 min) and produces complete inactivation of PAO in peripheral organs of rats and mice in doses in excess of 20 mg/kg body wt. (Bolkenius & Seiler, 1984; Bolkenius *et al.*, 1984). In contrast with humans, rodents excrete putrescine mainly as such, not in the form of acetylputrescine (Seiler *et al.*, 1981b). By using AG and MDL 72521 it is therefore now possible to study the role of polyamine catabolism in polyamine excretion and establish a polyamine balance in intact animals.

Materials and methods

Chemicals

Usual laboratory chemicals were from E. Merck (Darmstadt, Germany) or Baker Chemicals (Deventer, The Netherlands). Putrescine dihydro-

chloride, spermidine phosphate and spermine phosphate were from Fluka (Buchs, Switzerland). Octanesulphonate is a product of Eastman Kodak (Rochester, NY, U.S.A.) and *o*-phthalaldehyde of Carl Roth (Karlsruhe, Germany). Dowex 50W (X8; 200–400 mesh; Na⁺ form) was bought from Serva (Heidelberg, Germany).

AG was from Th. Schuchardt (Munich, Germany), α -DL-difluoromethylornithine (DFMO) was synthesized in our Institute according to a published procedure (Metcalf *et al.*, 1978), and MDL 72521 was synthesized as described by Bey *et al.* (1985).

Laboratory animals

Male Sprague–Dawley rats weighing at the beginning of the experiment 236 ± 10 g were from Charles River (St. Aubin-les-Elbeuf, France). They were housed singly in metabolic cages. Before the experiments were started they were adapted for 3 days to their environment (22°C, 60% relative humidity, 12h-light/12h-dark cycle, free access to powdered standard diet and water).

Treatment schedule and urine collection

Each treatment group consisted of three animals. One 24 h urine sample was collected from all rats the day before the first treatment with drugs. The control group received daily intraperitoneal injections of physiological saline (0.9% NaCl) (0.1 ml/100 g body wt.). AG (25 mg/kg), MDL 72521 (20 mg/kg) and combinations were given daily by intraperitoneal injection, the volume being again 0.1 ml/100 g body wt. DFMO was administered in the form of a 3% (w/v) solution instead of drinking water. The average daily drug intake was about 4 g/kg body wt.

Urine samples (24 h) were collected during the entire period in bottles containing 20 ml of ethanol. The volume of the individual samples was adjusted to 50 ml with ethanol, and the samples were stored in a refrigerator until analysis. At the end of the experimental period (24 h after the fifth treatment) the animals were decapitated. Organs were rapidly removed and homogenized with 10 vol. of 0.2 M-HClO₄. The HClO₄ extracts were submitted to polyamine analysis.

Determination of polyamines

Samples (100 μ l) of the urines were mixed with 800 μ l of 0.2 M-HClO₄ and with 100 μ l of a 5 μ M solution of heptane-1,7-diamine dihydrochloride (internal standard) in 0.2 M-HClO₄. Aliquots (200 μ l) were applied directly on the reversed-phase column with a loop injector.

Hydrolysates of the urines were prepared as follows. Aliquots (1 ml) were acidified with 0.1 ml of 1 M-HCl and evaporated to dryness. The residues

were dissolved in 0.5 ml of 6 M-HCl and the glass tubes were sealed and heated for 18 h at 120°C.

Samples (50 μ l) of the hydrolysates were diluted with 850 μ l of 0.2 M-HClO₄ and 100 μ l of heptane-1,7-diamine solution (see above). These diluted samples were subjected to h.p.l.c.

Recovery of polyamines after hydrolysis was estimated by hydrolysis of standard mixtures, and by hydrolysis of urine samples to which known amounts of acetyl derivatives were added. The mean recovery of putrescine was 94% and for spermidine, 63% (Seiler & Knödgen, 1979).

For the determination of the polyamines and their monoacetyl derivatives the previously described separation of their ion pairs with *n*-octanesulphonic acid on a μ Bondapak C₁₈ column (Waters, Paris, France), followed by reaction with *o*-phthalaldehyde/2-mercaptoethanol and fluorimetric evaluation of the resulting reaction products was used (Seiler & Knödgen, 1980).

Results

The results of the urinary polyamine analyses are summarized in Table 1. Polyamine excretion had stabilized during days 3–5 of treatment. Therefore mean values $\pm$ s.d. of the 24 h excretions are given for this period.

Urinary polyamine excretion by the saline-treated control group was fairly constant during the experimental period. The average weight gain of 22% (which was the same for all treated groups) had no significant effect on polyamine excretion. Similarly, all groups showed, in the pretreatment urine, very similar patterns.

The excretion of *N*⁸-acetylspermidine was not altered significantly by any treatment; spermine excretion by rats is very low, and was not increased significantly under the experimental conditions.

Daily administration of 20 mg of MDL 72521/kg body wt. did not change total putrescine excretion; however, the results were at greater variance than in control animals. *N*¹-Acetylspermidine excretion increased from about 0.2 to 3 μ mol/24 h, whereas spermidine excretion was not altered.

In contrast, administration of 25 mg of AG/day per kg body wt. produced a 3–5-fold increase in putrescine excretion, and small but significant increases in *N*¹-acetylspermidine and spermidine excretion. The most interesting results were those obtained after combined treatment with AG and MDL 72521: putrescine excretion was less enhanced than after administration of AG alone, but *N*¹-acetylspermidine excretion was even greater than after MDL 72521 alone.

Although it is known that *N*¹-acetylspermine is a natural compound that becomes detectable in tissues in situations of enhanced polyamine

Table 1. *Average daily polyamine excretion during days 3-5 of treatment*
 There were three rats per treatment group and three urine samples were taken per animal. Results are means $\pm$ s.d. Phys. sal., physiological saline.

Treatment*	Excretion (μ mol/24h per rat)				
	Polyamine ... Putrescine	Cadaverine	N ¹ -Acetyl-spermidine	N ⁸ -Acetyl-spermidine	Spermidine
(a) Non-hydrolysed urines					
Phys. sal.	2.4 $\pm$ 0.3	0.4 $\pm$ 0.05	0.24 $\pm$ 0.03	0.11 $\pm$ 0.02	0.30 $\pm$ 0.07
MDL 72521	1.8 $\pm$ 0.7	0.4 $\pm$ 0.1	3.1 $\pm$ 0.4†	0.10 $\pm$ 0.01	0.2 $\pm$ 0.1
AG	6.6 $\pm$ 1.4†	2.9 $\pm$ 0.7†	0.4 $\pm$ 0.1	0.12 $\pm$ 0.03	0.5 $\pm$ 0.2
AG + MDL 72521	4.7 $\pm$ 1.1†	3.0 $\pm$ 0.2†	4.3 $\pm$ 0.5†	0.09 $\pm$ 0.01	0.5 $\pm$ 0.3
AG + DMFO	1.2 $\pm$ 0.1†	2.4 $\pm$ 0.3†	0.22 $\pm$ 0.04	0.09 $\pm$ 0.01	0.24 $\pm$ 0.04
AG + MDL 72521 + DFMO	1.0 $\pm$ 0.1†	2.3 $\pm$ 0.4†	2.2 $\pm$ 0.2†	0.08 $\pm$ 0.01	0.31 $\pm$ 0.02
(b) Hydrolysed urines†					
Phys. sal.	2.0 $\pm$ 0.2 (83)	0.35 $\pm$ 0.03 (88)			0.60 $\pm$ 0.09 (86)
MDL 72521	1.7 $\pm$ 0.1 (94)	0.39 $\pm$ 0.09 (98)			2.4 $\pm$ 0.1 (71)†
AG	5.5 $\pm$ 0.5 (83)†	2.8 $\pm$ 0.2 (97)†			0.83 $\pm$ 0.02 (75)
AG + MDL 72521	4.0 $\pm$ 0.1 (85)†	2.5 $\pm$ 0.1 (83)†			3.3 $\pm$ 0.2 (67)†
AG + DFMO	1.3 $\pm$ 0.2 (108)†	2.0 $\pm$ 0.2 (83)†			0.41 $\pm$ 0.05 (68)
AG + MDL 72521 + DFMO	1.2 $\pm$ 0.3 (120)†	2.0 $\pm$ 0.2 (87)†			1.7 $\pm$ 0.2 (65)†
					2.9 $\pm$ 0.4 (81)
					3.1 $\pm$ 0.4
					5.2 $\pm$ 1.2†
					7.6 $\pm$ 1.7†
					9.6 $\pm$ 1.8†
					1.8 $\pm$ 0.2†
					3.6 $\pm$ 0.4
					0.7 $\pm$ 0.1
					3.4 $\pm$ 0.5†
					1.1 $\pm$ 0.3
					4.9 $\pm$ 0.7†
					0.6 $\pm$ 0.1
					2.6 $\pm$ 0.3†
					Total spermidine
					Total putrescine equivalents

* MDL 72521 (20 mg/kg), AG (25 mg/kg) and combinations were administered on five consecutive days by intraperitoneal injection: DFMO was given as a 3% solution in tap water.

† Values were not corrected for losses during hydrolysis; the percentage of the corresponding value obtained from non-hydrolysed urine is given in parentheses.

‡ Statistically significant ($P \leq 0.05$) difference between drug-treated and physiological-saline-treated groups.

degradation (Seiler *et al.*, 1981c), it was not detected in the urine of rats, irrespective whether they were treated with AG, MDL 72521 or the combination of the two inhibitors.

As would be expected from the treatment with an inhibitor of ornithine decarboxylase (ODC), the excretion of polyamines, especially that of putrescine, was greatly diminished in the rats that received 3% solution of DFMO as drinking fluid. If, however, MDL 72521 was administered in addition to DFMO, a very substantial increase of *N*¹-acetylspermidine excretion was produced that was only somewhat smaller than that in animals treated with MDL 72521 alone.

The effects of the same treatments on cadaverine excretion differed mainly in two aspects: neither the administration of the PAO inhibitor MDL 72521 nor that of DFMO had any significant influence on cadaverine excretion.

In order to test whether considerable amounts of polyamine conjugates are excreted that are not detected by the measurement of putrescine, spermidine, *N*¹-acetylspermidine and *N*⁸-acetylspermidine, urine samples hydrolysed with 6M-HCl were analysed. The results obtained from hydrolysed and non-hydrolysed urine samples, which were collected from day 3 to day 5 of treatment are compared in Table 1.

It appears from the results shown in this Table that there were losses of polyamines due to hydrolysis. The average recovery of total polyamines (putrescine equivalents) was 83%. In the DFMO-treated groups putrescine recovery (in contrast with recovery of cadaverine) was higher than in the saline-treated control animals and the other treatment groups. There are two potential explanations for this finding: either a product was formed from DFMO during hydrolysis that was co-eluted with putrescine from the reversed-phase column, or a conjugate of putrescine was excreted under these conditions. A specific study will be necessary to clarify this point. Since this finding does not greatly affect the interpretation of the results, and since it appears that the data obtained from the non-hydrolysed urines give, within the error of the method, a correct picture of the drug effects on the urinary polyamine pattern, they will be used in the following quantitative considerations.

In order to see whether treatments of rats with inhibitors of the catabolic pathways, or with DFMO, produce significant changes of the polyamines in tissues, liver, kidney, muscle and pancreas were analysed. It appears from Table 2 that administration of MDL 72521 produced a small but significant increase in *N*¹-acetylspermidine concentrations, and administration of DFMO was followed by a small decrease in total

polyamines in liver and kidney. But there was only a slight accumulation of polyamines after treatment with AG, demonstrating that the polyamines which are normally catabolized are excreted to a considerable extent if CuAO enzymes are inhibited.

Discussion

The enhanced excretion of putrescine and (total) spermidine after treatment of rats with AG was previously shown by Rojansky *et al.* (1979). It appears, however, that enhancement of *N*¹-acetylspermidine and of spermidine excretion is characteristic of AG-treated rats. This, and the fact that *N*¹-acetylspermidine excretion by MDL 72521-treated rats is further increased if the animals receive at the same time AG, is additional support to the notion (Seiler *et al.*, 1983) that *N*¹-acetylspermidine is a natural substrate not only of PAO, but also of CuAO, and is obviously degraded by this enzyme if it is formed in excess.

From the data in Table 1 one can conclude that only about 40% of the polyamines destined to be eliminated appear in the urine of the rats: 60% are catabolized by CuAO. Corresponding data for humans are not available at present, but a situation comparable with that in rats can be expected in humans as well. Both physiological (pregnancy) and pathological conditions, and treatment with certain drugs, may affect the activities of the oxidases that are involved in polyamine catabolism, and thus influence urinary polyamine patterns. If polyamine excretion is used for diagnostic purposes, it seems imperative to take polyamine degradation into consideration. This was not the case up until the present time (Scalabrino & Ferioli, 1982; Russell, 1983).

Inhibition of PAO by MDL 72521 had no effect on the urinary cadaverine. Administration of AG increased, according to the expectations, the excretion of cadaverine, but simultaneous treatment with DFMO did not decrease its excretion significantly. There is evidence that lysine can be decarboxylated by ODC, and that ODC is responsible for cadaverine formation in vertebrate organs (Pegg & McGill, 1979). It is, therefore, clear that either most of the urinary cadaverine is derived from a metabolic compartment that is not accessible to DFMO, or, more likely, it is formed by intestinal bacteria. Therefore, cadaverine must be excluded from considerations of polyamine balance.

By analogy to the formation of cadaverine from lysine by bacteria, we have to assume that a certain proportion of the residual putrescine that is found in the urine of DFMO-treated animals is also of bacterial origin. Precise values for this proportion

Table 2. *Polyamines in some organs of rats after 5 days of treatment*
Results are means $\pm$ S.D. ($n = 3$).

Treatment*	Concn. (nmol/g wet wt.)					Total putrescine equivalents
	Polyamine	Putrescine	N ¹ -Acetyl- spermidine	Spermidine	Spermine	
(a) Liver						
Phys. sal.†		10 ± 1	b.d.l.	1040 ± 70	800 ± 60	1850 ± 130
MDL 72521		11 ± 3	4 ± 0.5§	860 ± 70	680 ± 90	1560 ± 160
AG		30 ± 3§	b.d.l.	1080 ± 60	740 ± 40	1850 ± 100
AG + MDL 72521		11 ± 1	5 ± 0.7§	720 ± 100	730 ± 120	1470 ± 220
AG + DFMO		7 ± 2	b.d.l.	720 ± 90§	840 ± 40	1570 ± 130
AG + MDL 72521 + DFMO		6 ± 1§	3 ± 0.4§	660 ± 30§	900 ± 10	1570 ± 40
(b) Kidney						
Phys. sal.		18 ± 1	b.d.l.	520 ± 90	840 ± 50	1380 ± 140
MDL 72521		13 ± 1	7 ± 0.0§	430 ± 40	860 ± 50	1310 ± 90
AG		49 ± 10§	b.d.l.	510 ± 40	850 ± 50	1410 ± 100
AG + MDL 72521		25 ± 4	9 ± 0.6§	420 ± 60	900 ± 10	1350 ± 80
AG + DFMO		14 ± 2	b.d.l.	300 ± 30§	770 ± 40	1080 ± 40§
AG + MDL 72521 + DFMO		12 ± 1§	5 ± 0.6§	290 ± 30§	880 ± 10	1190 ± 40§
(c) Muscle (leg)						
Phys. sal.		5 ± 1	b.d.l.	70 ± 9	240 ± 10	320 ± 20
MDL 72521		4 ± 0	2 ± 0.0§	64 ± 9	240 ± 10	320 ± 20
AG		9 ± 1§	b.d.l.	82 ± 12	230 ± 10	320 ± 20
AG + MDL 72521		5 ± 1	1 ± 1§	64 ± 9	240 ± 10	310 ± 20
AG + DFMO		3 ± 0	b.d.l.	62 ± 8	240 ± 9	310 ± 20
AG + MDL 72521 + DFMO		2 ± 1§	2 ± 1§	61 ± 10	250 ± 6	320 ± 20
(d) Pancreas						
Phys. sal.		28 ± 3	b.d.l.	5100 ± 190	730 ± 110	5860 ± 300
MDL 72521		23 ± 2	9 ± 1§	5240 ± 440	870 ± 160	6140 ± 600
AG		39 ± 13§	b.d.l.	4570 ± 170	680 ± 40	5290 ± 220
AG + MDL 72521		21 ± 0	8 ± 2§	4390 ± 1100	760 ± 170	5180 ± 1270
AG + DFMO		20 ± 3	b.d.l.	4620 ± 680	770 ± 150	5410 ± 830
AG + MDL 72521 + DFMO		15 ± 4§	10 ± 3§	4240 ± 550	840 ± 160	5110 ± 720

* MDL 72521 (20mg/kg), AG (25mg/kg) and combinations were administered on 5 consecutive days by intra-peritoneal injection; DFMO was given as a 3% solution in tap water; animals were killed 24h after the last dose.

† Phys. sal., physiological saline.

‡ b.d.l., below detection limit of the analytical method.

§ Statistically significant ($P \leq 0.05$) difference between drug-treated and physiological-saline-treated groups.

are, however, not available, since it is not possible to inhibit tissue ODC *in vivo* completely.

The effect of administration of MDL 72521 on the urinary polyamine pattern of rats is in accordance with an important role of polyamine interconversion, i.e. of putrescine formation from spermidine, and spermidine formation from spermine. Polyamine interconversion has previously been demonstrated by injection of labelled (Siimes, 1967) or non-labelled (Matsui *et al.*, 1982) polyamines. However, one can argue against this type of experiments that the exogenously administered compounds are at least initially not in equilibrium with the endogenous pools, and may, therefore, be exposed to catabolic reactions to an extent that differs considerably from that to which the endogenous compounds are exposed. In

addition, exogenous polyamines, if given in large amounts, induce polyamine *N*¹-acetylation (Matsui *et al.*, 1982), and it is therefore not possible to obtain physiologically relevant data.

It is known from long-term treatments of mice that MDL 72521 does not produce any toxic effects, even if given in amounts that produce a complete blockade of PAO activity in the peripheral organs. Furthermore, the activity of polyamine-synthetic enzymes (ODC and S-adenosylmethionine decarboxylase) and of acetyl-CoA:polyamine *N*¹-acetyltransferase were not changed to a measurable extent in the liver of mice treated with MDL 72521 (Bolkenius & Seiler, 1984). One may therefore assume that the results obtained in the present work reflect the physiological situation.

The increase of N^1 -acetylspermidine excretion from 0.24 to 3.1 $\mu\text{mol}/24\text{h}$ after treatment with MDL 72521 suggests a very substantial formation of putrescine from spermidine in the intact rat.

The comparison of total urinary polyamine excretion reveals an interesting fact: those animals which received MDL 72521 excrete around 2 μmol of putrescine equivalents more than the corresponding treatment group which did not receive the PAO inhibitor (Table 1). For example, control rats excreted 3.1 μmol of putrescine equivalents/day and MDL 72521-treated, 5.2 μmol ; AG-treated animals excreted 7.6 μmol , whereas AG + MDL 72521-treated excreted 9.6 μmol , again about 2 μmol of putrescine equivalents in excess. The excessive polyamine excretion occurred in the form of N^1 -acetylspermidine.

The explanation for this finding is simple if we assume that under physiological conditions a certain proportion of the interconversion-pathway-derived putrescine (2 $\mu\text{mol}/\text{day}$ per rat) is not excreted or catabolized, but is re-utilized for spermidine formation. Since this portion of putrescine cannot be formed in animals with inactivated PAO, it is excreted in the form of N^1 -acetylspermidine.

Re-utilization of putrescine for spermidine biosynthesis has previously been postulated to occur to a considerable extent in mouse brain (Antrup & Seiler, 1980). This postulate was based on the fact that the putrescine moiety of spermidine and spermine had apparently a considerably longer half-life than their aminopropyl moieties.

Even after repeated administration of MDL 72521, brain PAO activity is not completely inhibited (Bolkenius & Seiler, 1984), and therefore metabolites from brain should not greatly influence the urinary polyamine pattern. The re-utilization of putrescine must therefore be of considerable quantitative importance in peripheral organs as well, not only in brain.

The proportion of putrescine re-utilization does not seem to be diminished in animals with a greatly reduced ODC activity, as appears when the comparison is made between the urinary polyamine patterns of rats treated either with AG + DFMO, or with AG + DFMO + MDL 72521 (Table 1): although total polyamine excretion was diminished by 76%, owing to DFMO administration, additional treatment with MDL 72521 produced an increase of N^1 -acetylspermidine excretion similar to that observed in rats with intact ODC, that were treated only with AG + MDL 72521.

With AG, a potent inhibitor of CuAO, and the new PAO inhibitor, MDL 72521, we have now powerful tools in our hands that will allow us for the first time to obtain insights into the

quantitative significance of the two major catabolic pathways of the polyamines: terminal oxidation and interconversion. With the advent of these compounds it will be possible not only to study physiological situations, but also quantitative aspects of pathological and drug-induced alterations in polyamine metabolism.

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